A Parasite Reveals Cryptic Phylogeographic History of Its Host: The Colonization of the Western Mediterranean Islands

C. Nieberding[1,2], S. Morand[2], R. Libois[1] and J.R. Michaux[1,2]

[1]Unité de Recherches Zoogéographiques; Institut de Zoologie; Liège, Belgium.
[2]Centre de Biologie et de Gestion des Populations, Campus international Agropolis de Baillarguet CS 30016, Montferrier-sur-Lez, France

Summary

This study compares the colonisation patterns of the field mouse *Apodemus sylvaticus* (Muridae) and its parasite *Heligmosomoides polygyrus* (Heligmosomoidea) in 8 Western Mediterranean islands. Respectively 690 bp and 501 bp of the mitochondrial cytochrome b gene were sequenced in 44 *H. polygyrus* and 49 *A. sylvaticus*.

The results reveal the congruence between both species' insular Mediterranean colonisation and highlight the role of biological "magnifying glass" of the parasite in precising the continental origin of insular wood mice: both species colonised the Balearic islands and Porquerolles from Spain or Southern France, while Elba, Sardinia and Sicily were colonized from Central-South Italy and Corsica from northern Italy, and Crete from Greece. Finally, Sicily should be considered as a "hot spot" of intraspecific genetic biodiversity.

Introduction

The study on insular populations mainly focused on mammals and birds : islands are depauperate in species richness, in comparison with area of similar size on the nearby mainland [1]. At the intraspecific level, insular populations are characterized by a decrease in genetic variability and by modifications in life history traits that are referred as the insular syndrome. Less known is the case of insular parasites populations. [2], [3]

and [4] detected a loss of species richness in helminth fauna of micromammals species in the Mediterranean islands, correlated to island area and distance from mainland [4]. Furthermore, a broadening of the ecological niche of helminth parasites was also evidenced: [5] and [4] showed that host specificity of helminth parasites in rodents like *Mus musculus* and *Rattus rattus* is released on Mediterranean islands as compared to the nearby mainland.

In this context, the phylogeographic patterns of the woodmouse *A. sylvaticus* (Rodentia, Muridae), and of its direct and specific endoparasite, the Nematod *H. polygyrus* (Dujardin, 1831), were analysed in 8 Western Mediterranean islands. *H. polygyrus* is an *A. sylvaticus* direct and specific endoparasite. Host contamination occurs with ingestion of parasitized faeces [6]. *H. polygyrus* has high prevalence and abundance.

The results were analysed in comparison with those previously obtained on source-mainland populations: both species survived to the Quaternary ice cycles in three main Mediterranean refuge zones (Iberian, Italian and Balkan refuges). Following the last glacial maximum, western Europe was recolonised from the Iberian populations in both species, whereas Italian and Balkan animals remained blocked by the Alpine barrier as well as by their low population densities (for more details, see [7]; [8]).

The aims of this study are to assess the geographic origin, epoch and mode of colonization of insular populations for both species. The parasite was used as a biological magnifying glass as it puts into light more detailed hypotheses about its specific host colonization history in the Mediterranean islands.

Material and methods

A total of 44 *H. polygyrus* individuals and 49 *A. sylvaticus* individuals were sampled on respectively 7 -Corsica, Crete, Elba, Mallorca, Menorca, Sardinia and Sicily- and 8 –Porquerolles in addition- Mediterranean islands. These samples were analysed with bordering continental Mediterranean populations of both species [7]; [8]. *H. polygyrus* and *A. sylvaticus* DNA extraction, amplification and sequencing of 687 bp and 501 bp of cytochrome b respectively were carried out as described in [8].

The *H. polygyrus* data matrix comprised 107 sequences as well as *Heligmosomum costellatum* (Dujardin, 1845) used as outgroup. The *A. sylvaticus* data matrix comprised 115 sequences as well as 2 *A. flavicollis* used as outgroups. *H. polygyrus* data were analysed by distance (Neighbor Joining, NJ) and Maximum Parsimony (MP) using PAUP 4.0b8 package [9]. The robustness of inferences was assessed by bootstrap resampling (BP) [10] using 1000 random NJ and MP repetitions.

Nucleotide (n) and haplotype (π) diversities were calculated using the

DnaSP 4.0 program [11]. Genetic divergence values between the groups of samples were obtained using a distance analysis (K_2P distance estimator).

Results

Phylogenetic relationships of sequences

For *A. sylvaticus*, the islands of Mallorca (Ma1), Menorca (Mi1), Porquerolles (Po1) fit into the Western European clade (Fig.1). Alternatively, the populations of Crete (Cr1), Corsica (Co1), Elba (El1) and Sardinia (Sa1) are comprised in the Italo-Balkan clade. None of these islands form distinct clades but are dispersed within the two main continental groups. In contrast, the Sicilian populations (Si1 and Si2) form a distinct clade well supported by BP, and are the sister clade of the Italo-Balkan one.

The parasite populations of Mallorca (Fig.2) fit into the Spanish endemic clade. This is also the case of the majority of Menorca sequences. Alternatively, the populations of Crete form part of the Balkan clade. Corsica, Elba, Sardinia and Sicily are comprised in the Italian clade: the Sardinian haplotypes, as well as the Elba and the Sicilian populations form part of the southern Italian subclade, while the Corsican population is contained in the Northern Italian one. Moreover, Corsica and Sardinia do not form distinct clades but are dispersed within the main continental groups. In contrast, the Sicilian populations form a distinct clade well supported by BP; the same is true for Elba at the exception of one sequence.

Genetic divergence, nucleotide and haplotype diversities

π and h diversities, and mean GD were calculated for insular populations in both species and

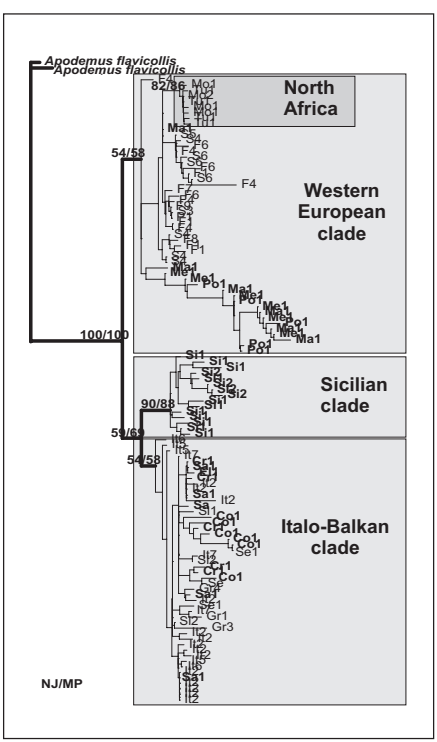

Figure 1: Consensus phylogenetic tree of *A. sylvaticus* sequences represented by their geographic origins (see text), and *A. flavicollis* used as outgroup. Numbers on branches indicate, from left to right, bootstrap support obtained in the NJ reconstruction (GTR+I+G)

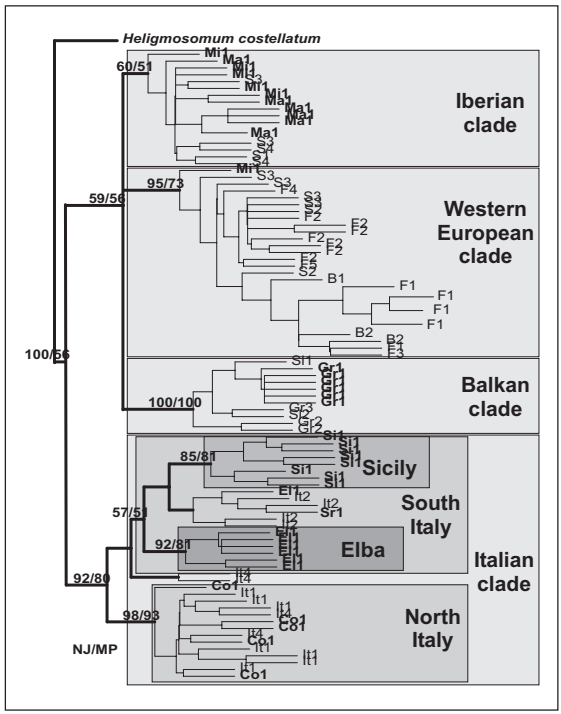

Figure 2.

compared with values obtained for the continental populations (Table 1), to estimate the loss of genetic variability of insular populations.

For *A. sylvaticus*, Sicily, Corsica and Porquerolles exhibit high values of ð and GD, while h values remained equivalent (Table 1). Crete displays diversities values of the same order of magnitude than continental ones. In contrast, Sardinia presents a crash of genetic diversity for π, h and GD values. For *H. polygyrus*, Sicily displays high values of genetic diversity. Corsica, Elba and Menorca present intermediate diversities values, while a crash of genetic diversity is observed in the islands of Crete and Mallorca.

Mean GD was also estimated between insular and source-mainland populations for both species, to estimate the importance of insular populations differentiation from source-mainland (Table 2). Mallorca, Menorca and Porquerolles were compared to western European continental ones (Spain, Portugal and France), while the islands of Corsica, Elba, Sardinia and Sicily were compared respectively to the Italian clade of *H. polygyrus* or to the Italo-Balkan clade of the host. Genetic differentiation of Crete was estimated by comparison with the Balkan clade of *H. polygyrus* and the Italo-Balkan clade of *A. sylvaticus*.

In *A. sylvaticus*, Sardinia and Elba present very low genetic differentiation. In contrast, the genetic differentiation of Sicily is high. Finally, the case of Corsica is intermediate. In *H. polygyrus* data set, the closer phylogenetic relationship of both Balearic islands with the Iberian endemic clade rather than with the western European ones was confirmed. Corsican populations are linked to the Northern Italian subclade, while the Sardinian and Sicilian populations rather belong to the southern Italian subclade. The case of Elba is more a near thing : a slight signal is found to join Elba to the southern Italian clade rather to the

Islands	*H. polygyrus* **(690 bp)**				
	Nber Ind	Nber Ht	GD (%K2P) (SD)	Pi (SD)	h (SD)
Corsica	6	5	1.88 (0.01)	0.019 (0.003)	0.933 (0.122)
Sardinia	1	1	_	_	_
Elba	6	10	1.57 (0.01)	0.013 (0.005)	1.0 (0.045)
Sicily	10	10	2.57 (0.01)	0.021 (0.002)	1.0 (0.045)
Ibiza	_	_	_	_	_
Mallorca	7	5	0.67 (0.01)	0.005 (0.001)	0.844 (0.080)
Menorca	7	5	1.43 (0.02)	0.018 (0.006)	0.667 (0.163)
Porquerolles	_	_	_	_	_
Crete	7	7	0.47 (0.02)	0.004 (0.001)	0.911 (0.077)
Spain	14	12	1.99 (0.02)	0.018 (0.006)	0.995 (0.034)
Western Europe	14	13	2.50 (0.02)	0.023 (0.005)	0.997 (0.023)
Italy	22	21	2.59 (0.01)	0.024 (0.003)	0.996 (0.015)
Balkans	13	13	1.79 (0.01)	0.017 (0.001)	1.0 (0.030)

Islands	*A. sylvaticus* **(501 bp)**				
	Nber Ind	Nber Ht	GD (%K2P) (SD)	Pi (SD)	h (SD)
Corsica	6	4	1.52 (0.01)	0.011 (0.003)	0.800 (0.172)
Sardinia	5	2	0.16 (0.01)	0.002 (0.001)	0.400 (0.237)
Elba	1	1	_	_	_
Sicily	15	15	1.67 (0.01)	0.016 (0.002)	1.0 (0.001)
Ibiza	_	_	_	_	_
Mallorca	6	6	_	_	_
Menorca	6	1	-	_	_
Porquerolles	5	4	1.47 (0.01)	0.010 (0.003)	0.900 (0.161)
Crete	5	5	1.12 (0.01)	0.011 (0.003)	1.0 (0.016)
Western Europe	26	23	1.19 (0.01)	0.010 (0.001)	0.950 (0.011)
North Africa	8	7	0.35 (0.01)	0.003 (0.001)	0.964 (0.077)
Italy-Balkans	32	18	0.95 (0.01)	0.006 (0.001)	0.881 (0.052)

Table 1: Mean genetic diversity observed among *H. polygyrus* (up) and *A. sylvaticus* (down) within islands for : GD for genetic divergence (in K_2P distance); Pi for nucleotide diversity and h for haplotype diversity. SD = Standard deviation.

A. sylvaticus	**Menorca**	**Mallorca**	**Porquerolles**		
Western Europe	4.29 (0.02)	4.13 (0.02)	4.57 (0.01)		
	Corsica	**Sardinia**	**Elba**	**Sicily**	**Crete**
Italy-Balkans	2.44 (0.01)	0.68 (0.01)	0.80 (0.01)	3.62 (0.01)	1.08 (0.01)

H. polygyrus	**Menorca**	**Mallorca**			
Iberia	1.66 (0.01)	1.25 (0.01)			
Western Europe	4.46 (0.02)	4.00 (0.02)			
	Corsica	**Sardinia**	**Elba**	**Sicily**	**Crete**
North Italy	1.72 (0.01)	5.27 (0.01)	3.87 (0.01)	4.48 (0.01)	
South Italy	4.32 (0.01)	4.36 (0.01)	3.21 (0.01)	3.47 (0.01)	
Balkans					1.40 (0.01)

Table 2: Mean genetic differentiation (in K_2P distance) observed among *A. sylvaticus* (up) and *H. polygyrus* (down) insular populations and source-mainland ones. SD = Standard deviation.

Northern Italian one. The genetic differentiation of Sardinia, Sicily and Elba from mainland populations appears important, while the one of Corsica is more tenuous. Finally, the Crete insular population is not differentiated from continental Greek ones.

Discussion

Archeozoological data show that colonization of Mediterranean islands by different human Neolithic cultures provoked the arrival of modern continental species, particularly of *A. sylvaticus*. However, paleontological and archeozoological data can not help to assess the origin of the first colonizers. In this context, this study shows that both species colonized the Balearics and Porquerolles from South western Europe, the Tyrrhenian islands and Sicily from continental Italy and Crete from Greece.

However, the existence of more structured geographic and genetic groups in *H. polygyrus* phylogeographic continental pattern allows the parasite to highlight more precisely the colonization history of *A. sylvaticus* and itself in these islands. Indeed:

- Balearic islands populations of *H. polygyrus* are nested with the Iberian clade rather than with the south western one; therefore, the insular population of both species are coming from Spain rather than Southern France.
- The study of cytochrome b gene in *A. sylvaticus* does not allow to

distinguish Italian from Balkan rodent populations Parasite phylogeographic reconstructions attest that colonisation of the Tyrrhenian islands originates from continental Italy. Therefore, the use of *H. polygyrus* data set allows to exclude the hypothesis of Balkan colonization of the Tyrrhenian islands at the epoch of Greek commercial trade with Mediterranean islands (2500 BP)

- Moreover, some archaeological, RFLP and enzymes studies on *A. sylvaticus* proposed that colonization of Corsica had occurred via Elba; and the colonization of Sardinia via Corsica [12]. However, the differentiation of northern and southern Italy in *H. polygyrus* (north and south of Sienna), shows that *H. polygyrus* Corsican populations are linked to the northern Italian subclade, while Elba and Sardinia form part of the southern Italian subclade. Furthermore, parasites from Elba form a distinct subclade within the southern Italian one and does not contain the Sardinian sequence. Therefore, the colonization of Corsica, Sardinia and Elba were independent events. The differentiation of Elban parasite population cannot be confirmed by the host data set due to a lack of sampling in this island; nevertheless, it confirms the particular history of the mice settlement on this island in comparison with Corsica and Sardinia. Moreover, the colonization of the central and southern parts of Sardinia, invaded by Iberian navigators through the Balearics, did not influence the Italian origin of *A. sylvaticus* and of its parasite populations.
- This study confirms the southern continental Italian origin of Sicilian populations of both species and the role of "hot spot" of biodiversity of this island (high diversities values and important differentiation from source-mainland). Indeed, several connections between Sicily and Italy existed for the last MY and southern Italy was isolated several times by the sea from the northern part of the country (Jaeger, Pers. Comm.; for more details, see [8] ; [13].
- The colonisation of Crete by both species took its origin in Greek mainland. This result is totally different as compared to other small mammal species living in this island. Indeed, *A. mystacinus*, the shrew *Crocidura suaveolens* as well as the spiny mouse *Acomys minous* invaded Crete from Turkey or the Near East [14]; [15]; Magnanou Pers. Com. The reason of the particular origin the wood mouse and *H. polygyrus* is to be associated with the absence of these species in Turkey and the Near East.

In conclusion, a previous study had highlighted synchronous co-differentiation of *H. polygyrus* and its host in Western Europe, Italy and Sicily [8]. In these regions, the parasite acts as a biological magnifying glass as it revealed previously undetected phylogeographic information in the host. The extension of the study of *H. polygyrus* phylogeographic pattern to 8 western Mediterranean islands confirms this role of biological magnifying glass.

References

1. Blondel J. Evolution and ecology of birds on islands: trends and prospects. Vie et Milieu;50:205-220 (2000).
2. Mas-Coma S, Esteban JG, Fuentes MV, Bargues MD, Valero MA, Galan-Puchades MT. Helminth parasites of small mammals (Insectivores and Rodents) on the Pityusic island of Formentera (Balearic Archipelago). Res. Rev. Parasitol.;58:125-133 (1998).
3. Mas-Coma S, Esteban JG, Fuentes MV, Bargues MD, Valero MA, Galan-Puchades MT. Helminth parasites of small mammals (Insectivores and Rodents) on the Pityusic island of Eivissa (Balearic Archipelago). Res. Rev. Parasitol.;60:41-49 (2000).
4. Goüy de Bellocq J, Morand S, Feliu C. Patterns of parasite species richness of Western Palearctic micro-mammals : island effects. Ecography;25:173-183 (2002).
5. Mas-Coma S, Feliu C. Helminthfauna from small mammals (insectivores and rodents) on the Pityusic Islands. In: Kuhbier H, Alcover, J. A., Guerau d'Arellano Tur (Eds.), ed. Biogeography and Ecology of the Pityusic Islands. The Hague, Boston, Lancaster: Dr W. Junk publishers, 1984.
6. Ehrenford FA. The life cycle of *Nematospiroides dubius* Baylis (Nematoda: Heligmosomidae). J. Parasitol.;40:480-481 (1954).
7. Michaux JR, Magnanou E, Paradis E, Nieberding C, Libois R. Mitochondrial phylogeography of the Woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region. Mol Ecol;12:685-97 (2003).
8. Nieberding C, Morand S, Libois R, Michaux JR. A parasite reveals cryptic phylogeographic history of its host. Proc R Soc Lond B Biol Sci in press.
9. Swofford DL. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b. Sunderland. Massachusetts, 1998.
10. Felsenstein J. Confidence limits on phylogenies with a molecular clock. Syst Zool;34:152-161 (1985).
11. Rozas J, Rozas R. DNASP, version 2.0 : a novel software package for extensive molecular population genetic analysis. Computer Application in the Biosciences;13:307-311 (1997).
12. Michaux JR, Filippucci MG, Libois RM, Fons R, Matagne RF. Biogeography and taxonomy of *Apodemus sylvaticus* (the woodmouse) in the tyrrhenian region: enzymatic variations and mitochondrial dna restriction pattern analysis. Heredity;76:267-277 (1996).
13. Nieberding C, Morand S, Douady CJ, Libois R, Michaux JR. Phylogeography of a Nematode (*Heligmosomoides polygyrus*) in the Western Palearctic region: Persistence of Northern cryptic populations during ice ages? Mol Ecol submitted.
14. Barome PO, Lymberakis P, Monnerot M, Gautun JC. Cytochrome b sequences reveal Acomys minous (Rodentia, Muridae) paraphyly and answer the question about the ancestral karyotype of Acomys dimidiatus. Mol Phylogenet Evol;18:37-46 (2001).
15. Michaux J, Bellinvia E, Lymberakis P. Taxonomy, evolutionary history and biogeography of the broad-toothed Field Mouse (*Apodemus mystacinus*) in the eastern Mediterranean area based on mitochondrial and nuclear genes. Biol. J. Linn. Soc. In Press.